



Review

Visiting eggplant from a biotechnological perspective: A review

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ABSTRACT

Eggplant is a member of family Solanaceae, and it is commonly cultivated in many parts of the world. Eggplant is susceptible to a number of biotic and abiotic stresses, therefore, there is a continuous demand of varieties with disease and insect pest resistance, better nutraceutical capacity, and adaption to climate change. Biotechnological approaches/tools have helped in the expansion of eggplant ideotype. In this direction, tissue culture techniques for organogenesis and somatic embryogenesis are standardized in eggplant. Also, plant transformation techniques like agrobacterium mediated gene transfer has been established in eggplant. Even if, the information of eggplant from a biotechnology perspective is increasing yet there is a lack of knowledge. Techniques like gene editing have not been tried in eggplant, further, eggplant is still remains unexplored from the molecular farming outlook. In this review, we compile the information regarding tissue culture, genetic engineering, and genome editing advancements so far accomplished in the eggplant.

1. Introduction

Solanum melongena L. a member of Solanaceae is generally cultivated as fruit vegetable in sub-tropical and tropical regions of the world. It is commonly known as eggplant, aubergine, guinea squash or brinjal (especially in the Indian subcontinent) (Daunay, 2008). Eggplant, is majorly being grown in China, India, Egypt, Iran, and Turkey, etc. In 2018, 1.87 million ha were cultivated in the world for a total production of 51.28 million tonnes, of which 62% and 24% of the world production were covered by China and India respectively (FAO, 2016). There is an extensive diversity in cultivated eggplant in terms of its biochemical, phenotypic and physiological traits (Taher et al., 2017). Recently, breeders have focused on the wild relatives of eggplant with conventional, biotechnological, and genomics-based approaches (Gramazio, 2018).

Eggplant has a capacity to regenerate from tissues (explant), this regeneration capacity of plants has revolutionized the use of plants for genetic engineering approaches (Lynch, 2014; Smith, 2013; Vasil and Thorpe, 2013). In case of vegetable crops breeding, techniques involving gene transfer through sexual and vegetative propagation are well established (Bisognin, 2011; Lei, 2010). The general aim is to introduce genetic diversity into existing plant populations, further, to select and develop superior plants that carrying genes for desired explicit traits this is majorly done by the conventional breeding techniques (Dennis et al., 2008). The application of these conventional or classical

techniques has resulted in significant accomplishments like achieving the yield targets for vegetable crops (Borlaug, 1983). With the rapid development and advancement of in genetic engineering techniques/methods, based on the knowledge of gene structure and function, the scope of vegetable improvement has been dramatically broadened (Dalal et al., 2006).

Genetic transformation technologies offer direct access to useful genes not previously accessible to plant breeders (Lusser et al., 2012). With the rapid advancement, improved genetic engineering techniques, can simultaneously transfer several genes as a single event (Puchta and Fauser, 2013). Genetic engineering offers the possibility of integrating a desirable gene from closely related plants (cisgenic) without associated deleterious genes (linkage drag) or from related species, which do not readily cross with the crop of interest or from unrelated species (transgenic) even in other taxonomic phyla (Jacobsen and Schouten, 2007; Moradpour and Abdullah, 2017). Eggplant is susceptible to a wide range of biotic and abiotic stresses; therefore, insertion of resistance genes, along with yield and fruit quality improvement, is one of the main concerns of conventional breeding and biotechnology (Daunay, 2008; Daunay and Hazra, 2012).

Although, in many species, the development of rapid, highly efficient transformation, regeneration systems are still in progress and thus represents a bottleneck in the development of stable high yielding transgenic plants (Nyaboga et al., 2014). Moreover, the use of genetic engineering in crop improvement requires successful development and

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deployment of transgenic plants (Mba et al., 2012). Because of the advances in genetic transformation and gene expression technologies, a rapid progress has been observed during the last decade for the use of genetic engineering for crop improvement in terms of different biotic, abiotic stresses and male-sterility systems (Chase, 2006; Marco et al., 2015; Sree and Rajam, 2015). In case of eggplant some reviews are published on biotechnology and genetic engineering lines, but, they are either old or they lack all the important information at one place (Collonnier et al., 2001; Kashyap et al., 2003; Magioli and Mansur, 2005). Therefore in this review article the use of biotechnology in eggplant improvement, in terms of plant regeneration, genetic transformation, and genome editing methods with their application to the eggplant as a milestone approaches for crop improvement has been presented.

2. Conventional breeding in eggplant

Although, eggplant wild relatives can be easily crossed with the cultivated eggplant (Plazas et al., 2016). But, interspecific crosses between *S. melongena* and the other related *Solanum* species bearing desirable agronomical traits, have sometimes been limited by sexual barriers (Behera and Singh, 2002). As eggplant, is susceptible to a wide range of biotic and abiotic stresses; therefore, insertion of resistance genes is, with yield and fruit quality improvement, one of the main concerns of conventional breeding and biotechnology. Till date, more than 25 wild species of genus *Solanum* were used for crossing with cultivated eggplant with a limited success rate (Rotino et al., 2014). Interspecific hybrids in eggplants act as rootstocks in order to protect plants from fungal and bacterial infestations (Rotino et al., 1997). Also, it was shown that *S. incanum* can easily cross with cultivated eggplant and the resultant interspecific hybrids are fertile (Ranil et al., 2017). Importantly the use of wild species also results in transferring genes or genomic regions with undesirable traits. These undesirable traits can be avoided with several generations of backcrossing. Traits like fruit size can be easily recovered with some generations of backcrossing (Kouassi et al., 2016).

Even though various techniques can yield successful fertile hybrids, but still, wild relatives need to be used with caution because they may transfer unfavourable traits (such as high susceptibility to *Colletotrichum gloeosporioides* and bitter taste, because of a high level of steroid saponin, found in *S. torvum* and *S. linneanum* respectively) to hybrids in addition to traits potentially useful for eggplant breeding (Kashyap et al., 2003). Overall, the use of conventional breeding for the improvement of eggplant is limited by several barriers such as crossing issues (pre and post fertilization) linkage drag, etc. (Zulkarnain et al., 2015). For overcoming the above-mentioned limitations of conventional breeding, transgenic technology can be used as for the genetic improvement of eggplant (Sharma et al., 2002). The schematic representation of different steps involved in conventional breeding, marker assisted breeding and genetic transformation is presented in Fig. 1.

3. Plant regeneration

Eggplant responds well to cell, tissue and organ culture (Kantharajah and Golegaonkar, 2004). Plants can be regenerated from the tissues of eggplant either through embryogenesis (Ammirato, 1983) or organogenesis (Litz, 1993). This ability of regeneration has allowed the use of biotechnology, particularly the genetic transformation, exploitation of somaclonal variation, haploidy, and somatic hybridization (Collonnier et al., 2001).

3.1. Organogenesis

Numerous meristematic zones are formed from longitudinal sections of leaf explant that subsequently converted into shoot buds (Mukherjee

et al., 1991). The direct regeneration potential differs with the explant type used on a well-defined MS medium. Different explants have been found to show a differential response to regeneration on culture media having different combinations of cytokinins and auxins (Sidhu et al., 2014b) (Table 1). Hypocotyl, cotyledon, root, leaf explant had the different morphogenetic potential for numbers of adventitious shoots on different combinations of plant growth regulators (PGRs) (Mir et al., 2008; Sharma and Rajam, 1995; Zhang et al., 2014) (Table 1). Regeneration is also affected by 'age' of the explant as younger leaves showed better organogenesis than mature ones (Franklin et al., 2004; Prakash et al., 2015). Optimum combinations and concentrations of auxins and cytokinin have been determined for producing a maximum number of regenerated shoots in different cultivars of eggplant in many studies (Farhad et al., 2015; Foo et al., 2018; Yesmin et al., 2018) (Table 1). The dual role of sugar as carbon and osmotic source is well known in shoot regeneration, and enhanced shoot regeneration efficiency was reported when low sugar concentrations were used (Mukherjee et al., 1991) (Table 1). Genetic stability studies using Random amplified polymorphic DNA analysis also have been carried to reveal the monomorphic nature and true-to-type clones (Mallaya and Ravishankar, 2013).

3.2. Somatic embryogenesis

An artificial process in which an embryo is derived from a single somatic cell is called somatic embryogenesis (SE). There are two routes for SE, first is direct embryogenesis in which the embryos are directly developed from the explant tissue in the absence of callus proliferation and second is indirect embryogenesis in which calluses are formed first and then embryos developed from callus itself (Horstman et al., 2017). For the first time, (Yamada et al., 1967) reported SE in eggplant from immature seed embryos cultured on MS medium supplemented with IAA. Effects of genotype, explant type and its age, and media composition on plant regeneration have been reported in various studies. Out of all these factors, the type of explant has been a most important factor for induction of somatic embryos in eggplant (Kantharajah and Golegaonkar, 2004). Different explant types such as immature seed embryo, hypocotyl, cotyledon, leaf, root have shown different potential for SE (Table 1). Effects of genotype and genotype–explant interaction on both organogenesis and somatic embryogenesis have been discussed by various studies presented in Table 1. Recently, various modified protocols were suggested for SE (Habib et al., 2016) and for organogenesis (Muthusamy et al., 2014; Robinson and Saranya, 2013; Yesmin et al., 2018) for the efficient regeneration of plantlets in different cultivars.

4. Overview of gene transfer methods

The main purpose of genetic transformation is to manipulate the genome of important crop plant species by inserting one or few genes at a time. Using this technique we can transfer the DNA segment from any organism which has our trait of interest into the target crop plant. Initially, the genetic transformation has been used in tobacco for transferring different genes (insect resistance, disease resistance, herbicide tolerance, stress tolerance) (Binns, 1983; Potrykus et al., 1985). The technique of particle bombardment, also known as microprojectile bombardment, biolistics and particle acceleration, has been proved to be a very effective and versatile way for integrating genes into plant genomes. The DNA containing tungsten or gold particles, referred to as microprojectiles are carried by a microprojectile and are accelerated into living plant cells (Christou, 1992; Twyman and Christou, 2004). In eggplant, different explants have been targeted for transformation using the biolistic approach in eggplant but the use of cotyledon as an explant was found advantageous over others in terms of regeneration efficiency and several buds per explant (Sidhu et al., 2014a). Electroporation is the method where electrical impulses of high field strength are used to

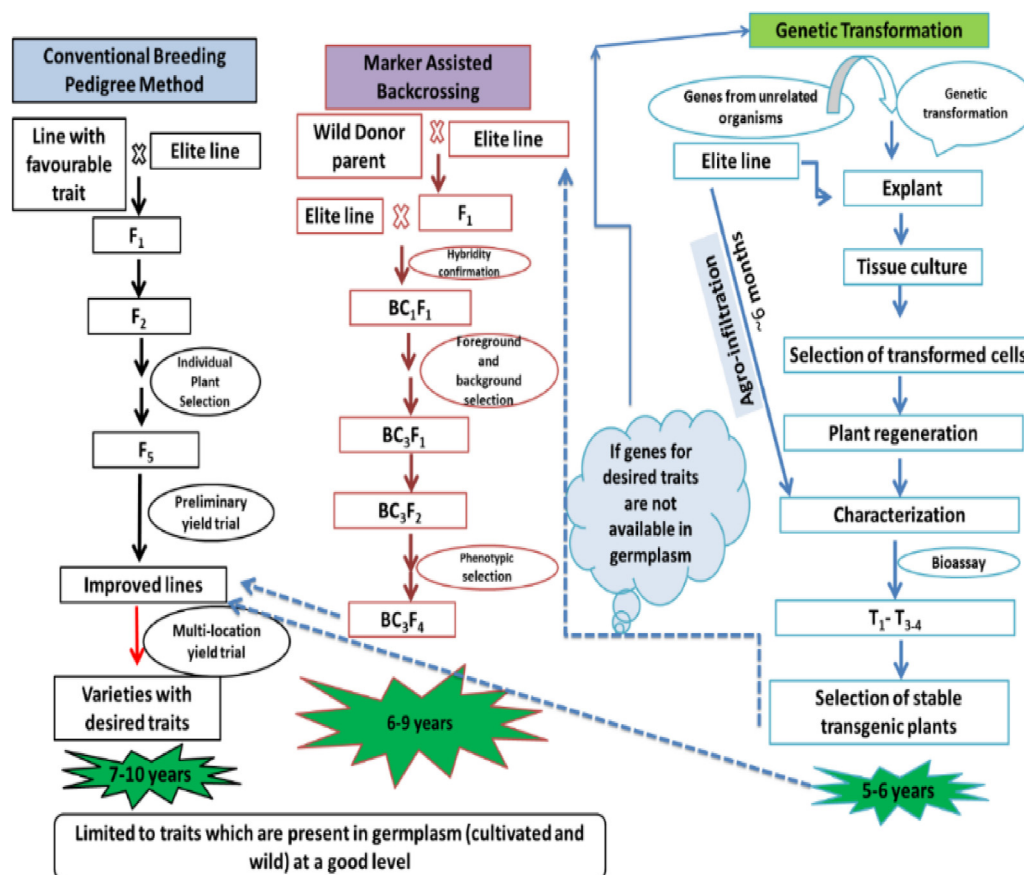


Fig. 1. A schematic representation of conventional breeding, marker-assisted backcrossing (MABC) and genetic transformation methods in eggplant improvement. Improved lines derived through genetic transformation can be either released as varieties or used as a donor parent in the MABC programmes.

reversibly permeabilize cell membranes to facilitate the uptake of DNA (Luft and Ketteler, 2015). This method has been used for a long time for transient and integrative transformation of protoplasts (Suginiura et al., 1999). Whereas, microinjection is the direct mechanical introduction of DNA under microscopical control into a cytoplasm or nucleus using a glass micro capillary-injection pipette (Chow et al., 2016). Although, there are several plant transformation methods, they are yet to be explored for the eggplant a schematic representation for steps involved in genetic transformation by different methods to generate transgenic plants is provided in Fig. 2.

4.1. *Agrobacterium tumefaciens* mediated gene transfer

Agrobacterium system was historically the first successful plant transformation system which revolutionized the field of plant genetic engineering. From then, *Agrobacterium tumefaciens* has been extensively used to introduce the gene into plant cells (Gelvin, 2003; Nester, 2015). The family Solanaceae of eggplant is well documented for its transformation efficiency (Van Eck, 2018). The first report of transformation in eggplant was in the late 1980s (Guri and Sink, 1988). Routinely, *agrobacterium* strains with binary vectors are employed to produce transgenic eggplants with kanamycin resistance (npt II) as the selective agent (Nester, 2015). The important aim of transformation in eggplant is to develop transgenic eggplant lines for resistance to Colorado potato beetle. In case, of eggplant different authors have developed regeneration and transformation procedures to transform eggplant (*Solanum melongena* L.) using seedling explants like hypocotyl, epicotyl, and node segments and cotyledon segments (Magioli and Mansur, 2005), leaf disks (Singh Yadav and Venkat Rajam, 1998), or roots (Franklin and Lakshmi Sita, 2003).

When an *Agrobacterium*-mediated transformation protocol was

used with cotyledons, hypocotyls and leaves from two eggplant genotypes high transformation efficiencies were reported (Kole et al., 2010). Influence of several factors such as type of explants, *Agrobacterium* cell density, infection time, pre-culture duration, co-cultivation duration, overgrowth control antibiotics on transformation efficiency also has been evaluated by various workers (Jayabalan, 2018; Prakash et al., 2016a). For example, (Habde et al., 2017) found maximum transformation efficiency when they used a two-week old cotyledon and three-week old hypocotyls explants precultured for 2 days and followed by infection with *Agrobacterium* suspension ($OD_{600} = 0.2-0.4$) for 1 min. Combination of cefotaxime and carbenicillin at 250 mg/L concentration was also found better for the effective control of *Agrobacterium* overgrowth. Jadhav et al. (2015) transformed four eggplant cultivars with cry1F Gene and produced transgenic plants having resistance against shoot and fruit borer.

4.2. Gene transfer by agroinfiltration

Grimsley et al. (1986) presented a method called, “Agroinfection” for the introduction of infectious viral DNA that uses the ability of *Agrobacterium* to transfer DNA from bacterial cells to plants. This combined use of the Ti plasmid and viral DNA has opened the way to molecular biological approaches that were not possible with either system alone. Recently, agroinfection by the croton yellow vein mosaic virus (CYVMV) of genus Begomovirus family Geminiviridae that has been achieved in tobacco and a replicon vector for expression of a foreign gene in plant was developed which can be used for studying the functional genomics, vaccine expression and gene silencing in plant (Jailani et al., 2016). This method is used for inducing the transient expression of genes in a plant organ, or isolated leaves, or even in cultures of plant cells (Leuzinger et al., 2013). In this method, firstly, a

Table 1
Plant regeneration in eggplant (*Solanum melongena* L.).

Explant	Cultivar	Growth regulators and their effects	Remarks	References
<i>Somatic embryogenesis</i>				
Immature embryo cultures				
Leaf	Imperial Black Beauty	IAA-Somatic embryogenesis (SE) 1.0 mg/L NAA-Maximal embryo yields 2:1 ($\text{NO}_3^-:\text{NH}_4^+$) – essential for embryogenesis	First time reported somatic embryogenesis in eggplant Cultivars showed significant quantitative differences in their capacities to form embryos	Yamada et al. (1967) Gledlie et al. (1983)
Leaf	F1 hybrid “Suphal”	8.0 mg/L NAA and 0.1 mg/L Kn – High Freq. SE	The frequency of plantlet regeneration varied from 27.0 to 49.7% in vitro and 2.0 to 4.5% in vivo.	Rao and Singh (1991)
Hypocotyl, epicotyl, leaf, cotyledon	F 100	54 μM NAA – induction of somatic embryos	The effects of position and orientation of the tissue on the culture medium were also studied	Magioli et al. (1998)
Cotyledon	Agroceres F-100 variety	54 μM NAA – induction of somatic embryogenesis	Prembryo formation was observed after the second day of culture	Tarré et al. (2004)
Hypocotyl, cotyledon and root	Punjab Barsati, Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93C	0.5 mg/L BAP + 1.0 to 2.0 mg/L NAA – Highest SE (hypocotyl, cotyledon, root) 0.25 mg/L BAP – regeneration into whole plants	Effects of genotype, explant and culture medium effects on SE were reported	Mir et al. (2008)
Hypocotyl	BARI Begun-1, BARI Begun-4, BARI Begun-5, BARI Begun-6 and Islampur	0.5 mg/L 2,4-D – development of embryogenic calli 1.0 mg/L 2,4-D – highest number of embryo (BARI Begun-4)	Optimized somatic embryogenesis protocol for locally adapted varieties in Bangladesh	Habib et al. (2016)
<i>Organogenesis</i>				
Excised hypocotyl segments	Cultivar and F1 hybrid	IAA 5.7 μM – 90% bud regeneration Combinations of IAA and BA or IAA and Z or IAA and SD8339 – 100% regeneration	Revealed that shoot differentiation is not essentially a function of cytokinin activity	Kamat and Rao (1978)
Hypocotyl	Fourteen commercial cultivars	0.8 mg/L NAA – only callus 0.016 mg/L NAA-adventitious roots 6BA-enhanced shoot production	Genotypic differences in response were observed	Matsuoka and Hinata (1979)
Stem pith	Florida Market	5.7 μM IAA and 4.5 μM each of 2,4-D and Kn – large aggregates	Suggested that some degradation product of ascorbic acid might be responsible for inducing leafy shoots.	Fassuliotis et al. (1981)
Hypocotyls, cotyledons and leaves	Six cultivars	2 mg/L IAA – callus and roots	Suggested that regenerative potential is under genetic control	Alicchio et al. (1982)
Leaf	Pusa Kranti	44 mM glucose or fructose – induction of shoot regeneration	Discussed the dual role of sugar as carbon and osmotic source in shoot regeneration	Mukherjee et al. (1991)
Hypocotyl, epicotyl, node, leaf and cotyledon	Brazilian eggplant variety (F-100)	0.2 μM TDZ-optimal shoot bud induction rates (75–100 buds/explant) 0.6 μM IAA – half strength-root induction	First report on the use of TDZ for in vitro regeneration of eggplant	Magioli et al. (1998)
Hypocotyl	Embu	0.25 mg/L IAA + 0.5 mg/L BAP-adventitious roots	Also evaluated influence of antibiotics (cefotaxime, timentin, kanamycin and hygromycin) on organogenesis	Picoli et al. (2002)
Isolated microspores	Hybrid Dsa	KM + 250 mg/L PEG, 0.2 mg/L 2,4-D, 0.5 mg/L ZT, 1 mg/L NAA-microspore culture	Showed that microspore dedifferentiation ability can be markedly improved by using the treatment of anther under the high temperature conditions	LianYong et al. (2004)
Intact root explants	MEBH 11, MEBH 9, Kalpatru, and Rohini	0.45 μM TDZ and 13.3 μM 6-BA – efficient regeneration	Observed negative correlation between regeneration and explant age	Franklin et al. (2004)
Cotyledons and true leaves	Two accessions of each gomba and scarlet	0.1 or 0.2 μM TDZ – efficient regeneration (70–100% explants with shoots)	Concluded that TDZ shows a high capacity for inducing organogenesis in African eggplants	Gisbert et al. (2006)
Cotyledon and midrib	Loda	2.0 mg/LNAA + 0.05 mg/L BAP – best callusing (83–85%)	Information from this study could be used as an alternative path to induce genetic and epigenetic changes in regenerated plants	Rahman et al. (2006)
Cotyledonary leaf, hypocotyl, shoot tip and root	Singhnath and Kazla	1.0 mg/L BAP and 1.0 mg/L Kn – high-frequency direct organogenesis of shoots	Demonstrated the efficiency of regeneration system by obtaining viable seeds from the regenerated plants	Sarker et al. (2006)
Cotyledons and under hypocotyls		3 mg/L 6BA – adventitious bud differentiation	Analyzed influence of auxin, genotype and explants on differentiation	Guang-yuan and Sha-sha (2007)
Microspore		0.2 mg/L 2,4-D, 0.5 mg/L Z + 1.0 mg/L NAA – callus induction	Observed significant genotypic differences for callus induction rate among the genotypes	Song et al. (2007)
Cotyledons and hypocotyls	Larga Negra and Black Beauty	2.0 mg/L NAA + 0.5 mg/L 6-BAP – callus induction	The highest per cent of callus induction was obtained from cotyledons	Zayova et al. (2012)
Shoot tip, hypocotyl and midrib	Jhumpy and Islampur	2 mg/L NAA – best for callus proliferation	Showed that it is possible to develop shoot and fruit borer tolerance brinjal genotypes through SE	Ferdiausi et al. (2009)
Microspore	Senyou 2 gou, Chikuyou and Hakata naganasu	0.4 mg/L 2,4-D + 0.2 mg/L BA – incubation of microspore induced calli	This improved method proved to increase the rate of plant regeneration from calli up to 70%	Saiki et al. (2009)
Cotyledon	Meizi, Xianfeng I, Heijuren and Jiuye	2.0 mg/L Zeatin + 0.1 mg/L IAA-bud induction	Analyzed genetic stability of the regenerated plants flow cytometry, RAPD and SSR molecular markers	Xing et al. (2010)
Cotyledon	Larga Negra	1.0 mg/L BAP – plant regeneration	Observed somaclonal variations among regenerated plants	Zayova et al. (2012)

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Table 1 (continued)

Explant	Cultivar	Growth regulators and their effects	Remarks	References
Stem, leaf and root	Jhunki	2.0 mg/L BAP and 0.5 mg/L NAA – highest callus induction	Suggested that the given protocol might be useful for the production of disease-free and healthy plant materials	Ray et al. (2010)
Cotyledonary leaf	Pusa Purple Long and Black Beauty	3 mg/L NAA + 0.5 mg/L Kn – max. callus induction	The plantlets showed high survival rate (80%) in the soil	Shivraj and Rao (2010)
Shoot tip and nodal explants	PKM-1	2.0 mg/L BAP + 1.0 mg/L 2iP – shoot proliferation	Efficient protocol was presented for micropropagation	Kanna and Jayabalan (2010)
Leaf	PLR	2 mg/L BAP + 1.0 mg/L 2iP – organogenic calli induction	Studied indirect organogenesis from leaf explants	Kanna and Jayabalan (2010)
Cotyledons, hypocotyls and leaf	Punjab Barsati, BSR 229 and KS 331	2.5 mg/L BAP – regeneration	Studied the effect of genotype and explants on in vitro plant regeneration	Charaya et al. (2011)
Hypocotyl, cotyledon and leaf	BL 5, BR 14, BSR 23	1.5 mg/L IBA + 1.0 mg/L BAP – 100% callusing and SE (cotyledon)	Cotyledonary callus of BSR 23 exhibited somatic embryos with highest regenerative potential	Kaur et al. (2011)
Hypocotyl, cotyledon and root	Punjab Barsati, Punjab Sadabahar, Jamuni Gola, PBSR-11 and BB-93C	2.5 mg/L IAA + 0.5 mg/L BAP – adventitious shoot induction	Genotype, explant and genotype × explant interaction showed highly significant effects on organogenesis	Mir et al. (2011)
Cotyledonary leave, nodal segment and shoot tip	Manjari Gota	2 mg/L 6-BAP + 1 mg/L Kn – best regeneration	Established in vitro rooted plantlets in polycarbonated polyhouse with 100% survival rate	Bhat et al. (2013)
Hypocotyls and cotyledon	Shisui	4.4 µM 6-BA + 0.2 µM TDZ – culturing of hypocotyl and cotyledon	Indicated that intact roots are important for explant shoot regeneration and development	Tanaka et al. (2013)
Tender shoot tip, hypocotyls, leaf and stem	Valuthalai	0.6 mg/L 2,4-D – max. proliferation	Presented an improved method for the in vitro propagation	Robinson and Saranya (2013)
Leaf, cotyledon and hypocotyl	Mattu Gulla and Perampalli Gulla	2.0 mg/L BAP + 0.5 mg/L IAA-shoot initiation	Developed an efficient and reproducible in vitro regeneration method	Muthusamy et al. (2014)
Leaf	Protab, Green Ball and Ghemma Begun	3 mg/L 2,4-D + 0.05 mg/L BAP – max. callusing	Studied the effect of genotypes and growth regulators on callus induction	Alim et al. (2015)
Hypocotyls and cotyledon	Four cultivars	2.0 mg/L Z + 1.0 mg/L 6-BA + 0.2 mg/L IAA – best callus induction	Showed that, the regeneration capacity of hypocotyls was significantly higher than that of cotyledons	Zhang et al. (2014)
Leaf	Protab, Green Ball and Ghemma Begun	1 mg/L NAA + 0.1 mg/L BAP – max. regeneration	The variety Protab appeared as the best for the shoot and root formation	Alim et al. (2015)
Microspore	F1 hybrid Bandera	0.2 mg/L IAA + 4 mg/L zeatin – shoot production	Regenerated doubled haploids from microspore-derived calli through organogenesis	Rivas-Sendra et al. (2015)
Hypocotyl	Manjarigota	2 µM BAP + 0.05 µM NAA – shoot regeneration	Assessed the effect of size, age and position of the explant, pre-culture and high cytokinin concentration in the pre-culture medium on shoot regeneration	Prakash et al. (2015)
Hypocotyl, cotyledon and leaf	BL-5, BR-14 and BSR-23	3.0 mg/L BAP – regeneration from cotyledon	Studied the effect of genotype, explant and culture media on direct plant regeneration	Kaur et al. (2015)
Cotyledon and leaf	PLR1	2 mg/L TDZ + 0.5 mg/L BAP + 0.5 mg/L NAA – shoot regeneration	Determined an efficient phytohormone concentration for development of organogenesis from explants	Taghipour et al. (2015)
Leaf		2.0 mg/L 2iP – max. shoot proliferation	Suggested to use this protocol for mass multiplication and also for regeneration of genetically transformed tissue	Kanna and Jayabalan (2015)
Hypocotyl	Nayantara, Kazla, Islampuri, ISD-006 and Uttara	2.0 mg/L ZR + 0.1 mg/L IAA – shoot regeneration	Established an efficient regenerating protocol for cultivated eggplant varieties	Muktadir et al. (2016)
Cotyledon	BARI begun-4 and BARI begun-6	1 mg/L BAP + 0.2 mg/L IAA – multiple shoot regeneration	The in vitro grown plantlets were acclimatized in soil, grew up to maturity, flowered, fruited and produced seeds as normal healthy plant like the control	Yesmin et al. (2018)
Cotyledon	Bulat Putih	2.0 mg/L Kn – shoot regeneration	Indicated that kinetin alone is sufficient to induce shoots without the presence of BAP	Foo et al. (2018)
<i>Both somatic embryogenesis and organogenesis</i>				
Cotyledons	Imperial Black Beauty	0.1–0.5 mg/L NAA – rhizogenesis	Suggested a role for polyamines in eggplant somatic embryogenesis	Fobert and Webb (1988)
Cotyledons	Nakate shinkuro	50/µM 2,4-D-embryogenic calli induction	Discussed the effects of an aseptic ventilative filter on somatic embryogenesis	Saito and Nishimura (1994)
Hypocotyl, cotyledon and leaf explants	Pusa Purple Long, Long White Cluster, Pusa Kranti, and Pusa Purple Cluster	11.1 µM BA + 2.9 µM IAA – optimum for shoot regeneration	Genotype, explant and genotype-explant interaction had highly significant effects on both organogenesis and somatic embryogenesis	Sharma and Rajam (1995)

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Table 1 (continued)

Explant	Cultivar	Growth regulators and their effects	Remarks	References
Cotyledon	Loda and China	0.05 mg/L BAP + 2.0 mg/L – best callus formation and SE	Developed an efficient and reliable method of indirect regeneration through SE	Huda et al. (2007)
Embryo from mature seeds, cotyledon and shoot explants	Thengathittu	10.6 mg/L NAA – callus production and SE (cotyledon)	Effects of various concentrations and combinations of NAA, Kinetin, 2,4-D, TDZ and BAP on cultures were studied	Swamynathan et al. (2010)
Hypocotyl, cotyledon and leaf	BSR-27, BR-16 and BL-7	1.5 mg/L IBA + 1.0 mg/L 6-BAP – highest SE	Studied the effect of media composition and explant type on the regeneration	Kaur et al. (2013b)

suspension of *A. tumefaciens* is introduced into a plant leaf either by direct injection or by vacuum infiltration or brought into association with plant cells immobilized on a support and then the bacteria transfer T-DNA into the plant cells (Han et al., 2015; Lee and Yang, 2006; Yang et al., 2000). Also, this might be quite useful to monitor the movement of elements like phosphorus (P) (Lin et al., 2016).

An improved protocol to enhance transformation efficiency of syringe agroinfiltration by combinative use of 5-azacytidine (AzaC), ascorbate acid (ASC) and Tween-20 was also developed using *N. benthamiana*. Further, suggested the optimal concentrations of AzaC (20 µM), ASC (0.56 mM) and Tween-20 (0.03%v/v) that significantly improved the transformation efficiency of agroinfiltration resulting into the increase in the expression of the transgene by over 6-fold (Zhao et al., 2017). This agroinfiltration process was optimized for the incubation period, proper infiltration medium and the optical density of the Agrobacterium suspension and evaluated in the eggplant leaves through transient expression of a reporter gene driven by a constitutive promoter (Kumar et al., 2017). Further, the efficiency of agroinfiltration-based transient gene expression was increased in *N. benthamiana*. A dual vector delivery system was developed by combining the most effective features (chemical additives, heat shock and the co-expression of genes) which provided approximately 3.5-fold higher levels of absolute GUS protein compared to the pEAQ-HT platform (Norkunas et al., 2018).

5. Marker-free transgenics

Selectable marker genes are needed for the selection of transformed cells. These are present in the vector along with the gene of interest and enable the transformed cells to survive on media containing toxic levels of selection agent while non-transformed cells get killed (Aragão and Brasileiro, 2002). Antibiotic, antimetabolite and herbicide resistance genes, hormone biosynthetic genes, etc., are the generally used selectable marker genes (Miki and McHugh, 2004). Presence of these genes in transgenic plants has been considered a serious problem for environment and the human, although, these selectable marker genes are usually not required once transgenic plants are produced (Maghari and Ardekani, 2011; Natarajan and Turna, 2007). A number of approaches have been proposed for the removal of these genes from transgenic plants viz. recombinase-based system such as recombinase flippase/flippase recognition target (FLP/FRT), Cre/lox, intra-chromosomal recombination system, transposable element-based system, co-transformation, Multi auto-transformation vector system (Hare and Chua, 2002; Scutt et al., 2002). Narendran (2006) produced marker-free insect-resistant transgenic plants for *Leucinodes orbonalis* expressing the cry2Ab gene by using *Agrobacterium*-mediated co-transformation. He used two plasmids, one containing cry2Ab gene and other having nptII and GUS marker genes and produced a large number of transgenic plants carrying both T-DNAs. Darwish et al. (2014) produced selectable marker-free transgenic eggplant resistant to *Alternaria solani* using the R/RS site-specific recombination system. They used Ipt gene as a selection marker gene and the gene of interest was Wasabi defensin (WD) gene. They confirmed the integration of the WD gene and excision of the selection marker (ipt gene) using molecular analyses of genomic DNA from transgenic plants.

6. Transgenic eggplant: resistant to stresses and other important traits

For the first time, Guri and Sink (1988) successfully transformed the eggplant using *Agrobacterium*-mediated genetic transformation with the cointegrate vector- pMON 200 harbouring nptII gene. This successful genetic transformation was then followed by a number of successful attempts to improve the transformation protocol and to produce the transgenics having desirable traits, using gene constructs with nptII as a selection marker and several reporter genes like gus (β-glucuronidase),

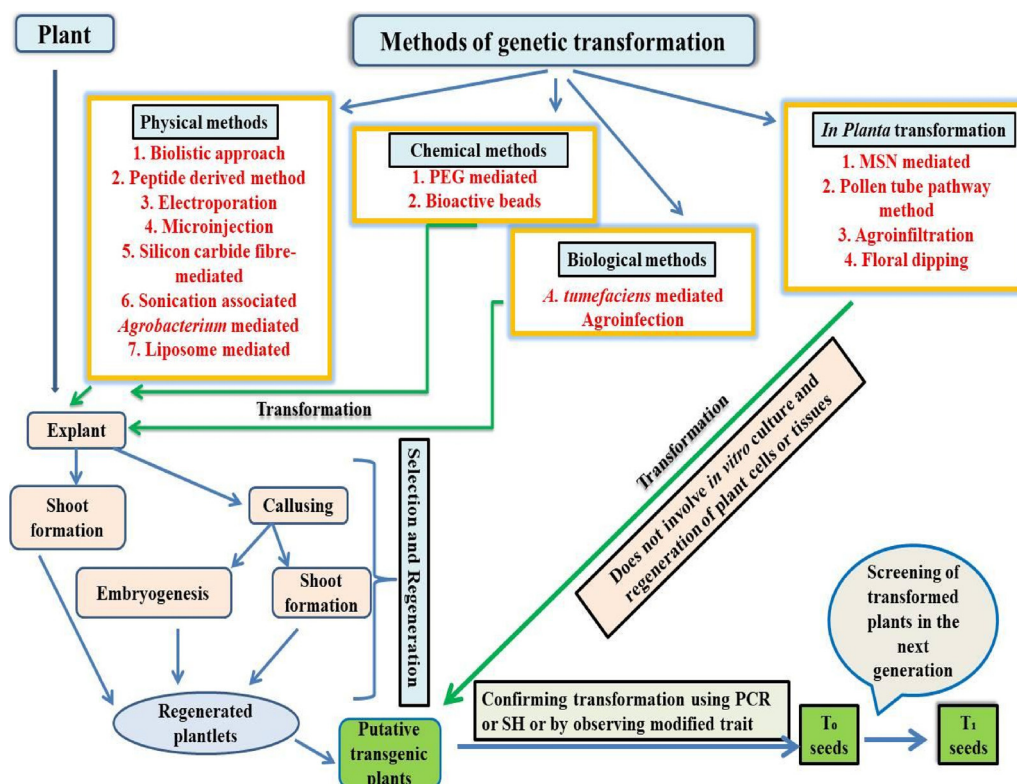


Fig. 2. Schematic representation for steps involved in genetic transformation by different methods to generate transgenic plants.

cat (chloramphenicol acetyl transferase) and *luciferase* (see Table 1). A number of studies have been conducted for increasing the efficiency of transformation protocol by optimizing the factors such as explant type, genotype and cultural conditions (Sunseri et al., 1993; Fári et al., 1995; Hanyu et al., 1999; Magioli et al., 2000; Franklin and Sita, 2003; Kannapiran, 2003; Prakash et al., 2016a; Hanur et al., 2016; Jayabalan, 2018). Transgenic plants resistance to colorado potato beetle (Arpaia et al., 1997; Hamilton et al., 1997; Jelenkovic et al., 1998), root knot nematode (Frijters et al., 2000; Goggin et al., 2006; Phap et al., 2010; Papolu et al., 2016), shoot and fruit borer (Kumar et al., 1998; Narendran, 2006; Pal et al., 2009), tomato chlorotic spot virus (Picoli et al., 2006), cucumber mosaic virus (Pratap et al., 2011), *Alternaria solani* (Darwish et al., 2014) and fungal wilts (Singh et al., 2015) using different genes from various sources, have been produced successfully.

Although, transgenic technology has been improved tremendously in eggplant and various studies have been carried out for optimizing the protocol of transformation for introducing the foreign genes, but there are only a few reports for producing the transgenic plants having tolerance to abiotic stresses (Prabhavathi et al., 2002; Prabhavathi and Rajam, 2007; Sagare and Mohanty, 2012; Kumar et al., 2014). In this direction, the first attempt was made by Prabhavathi et al. (2002), they introduced bacterial mannitol-1-phosphodehydrogenase (mtlD) gene into eggplant by *Agrobacterium tumefaciens*-mediated transformation. Produced transgenic lines were found to be tolerant against osmotic stress induced by salt, drought and chilling stress. In another study, a key polyamine biosynthetic gene arginine decarboxylase, *adc* was introduced into the eggplant with the aim to enhance stress tolerance in eggplant. The produced transgenic plants exhibited an increased tolerance levels to multiple abiotic stresses such as salinity, drought, low and high temperature, and heavy-metal and resistance against fungal wilt disease (Prabhavathi and Rajam, 2007). Sagare and Mohanty (2012) transformed eggplants with a binary vector pCAMBIA2300 carrying *rd29A::DREB1A* gene via *Agrobacterium* mediated transformation method and obtained moisture stress tolerant transgenic plants. In 2014, protocol for *Agrobacterium*-mediated gene transfer method

was further optimized and transgenic plants using foreign gene *HAL1* derived from yeast were produced. Different tests were carried out to evaluate the level of salt tolerance for the transgenic and control plants with culture media in vitro and with soil in greenhouse having various concentrations of salt, and demonstrated the possibility of increasing salt tolerance in eggplant (Kumar et al., 2014). Genetic engineering studies in eggplant need to be further exploited for the introduction of new genes encoding tolerance to various abiotic stresses and comprehensive studies to optimize factors that dramatically affect transformation (explant type, genotype and cultural conditions) are required (Table 2).

Some useful agronomically traits have also been transferred into eggplant using transgene technology (Rotino et al., 1997; Cao et al., 2010; Toppino et al., 2011; Padma, 2012). In this direction, first example comes from development of parthenocarpic fruits, this was achieved by expressing the coding region of the *iaaM* gene from *Pseudomonas syringae* pv. *savastanoi*, under the control of the regulatory sequences of the ovule-specific *DefH9* gene from *Antirrhinum majus*. This genetic manipulation in eggplant allowed fruit set and growth under environmental conditions prohibitive for fruit setting in the untransformed line, which did not set fruit at all; and production of fruits of marketable size from pollinated and un-pollinated transgenic flowers under normal environmental conditions (Rotino et al., 1997). Another useful study comes from Cao et al. (2010), they transformed the plants with *Barnase* and *Cre* genes separately for the production of male sterile lines and for restoring the fertility; and demonstrated the usefulness of *Cre/loxP* system, for heterosis breeding, producing hybrid seed of eggplant. Recently in 2012, *Agrobacterium* mediated transformation protocol was further optimized for the efficient production of transgenic eggplant and using this optimized protocol, plants were transformed with the *SmePPO1* gene in antisense orientation using pFGC1008 vector for reducing the expression level of *PPO* gene in fruits resulting into reduced enzymatic browning (Padma, 2012). In the future, it will be necessary to concentrate efforts on the production of specific secondary metabolites and the improvement of quality traits of

Table 2
Examples of agrobacterium mediated genetic engineering in eggplant for biotic, and abiotic stress tolerance along with other useful traits.

Explant (tissue/genotype)	Gene	Selectable marker gene	Trait	Remarks	Reference
<i>Biotic stress tolerance</i> Leaf sections/Black Beauty Callus cultures from leaves		NOS-NPT NPT + LUC		First successful genetic transformation The efficiency of transformation of <i>S. melongena</i> was lower than other <i>Solanaceae</i> species	Guri and Sink (1988) Komari (1989)
Cotyledonary and young leaves		NpIII		The highest proportion of transformed explants were obtained from intact cotyledonary leaf pieces	Filippone and Lurquin (1989)
Leaf/Picentia		NpIII		Development of a protocol for transformation using disarmed binary vector	Rotino and Gleddie (1990)
Picentia	Bt	NpIII NpIII	Resistance against Coleopterans	Low expression of toxin Developed a vivo method for screening of transgenic plants and observed Mendelian inheritance of Npt II	Rotino et al. (1992) Sunseri et al. (1993)
Cotyledons/Kecskemeti		NpIII		Developed a plant regeneration and genetic transformation protocol	Fáři et al. (1995)
Hypocotyl/Black Jack	Bt (CryIIIB)	NpIII	Coleopteran insect resistance	No significant resistance against the larvae of the Colorado potato beetle	Chen et al. (1995)
Leaf, cotyledon and hypocotyl/female parent of the hybrid 'Rimina'	Bt (CryIIIB)	NpIII	Colorado Potato Beetle resistance	Observed a significant insecticidal activity	Arpaia et al. (1997)
Shoot tip/Hibush	Bt (CryIIIB)	NpIII	Coleopteran insect resistance	Efficient protocol to increase the rate of transformation and regeneration	Billings et al. (1997)
Harris special	Bt (modified CryIIIA)	NpIII	Colorado Potato Beetle resistance	Transgenic lines against insect populations in the field at levels not significantly different from the insecticidal control	Hamilton et al. (1997)
Leaf segments/stock plants	Bt (synthetic CryIIIA)	NpIII	Colorado Potato Beetle resistance	Resistant to neonate larvae and adult Colorado potato beetle was observed	Jelenkovic et al. (1998)
Cotyledonary explants/Pusa Purple Long	Bt (synthetic cry1Ab)	NpIII	Fruit Borer (<i>Leucinodes orbonalis</i>) resistance	Proved that the synthetic gene based on monocot codon can be expressed in dicotyledonous plants for insect control	Kumar et al. (1998)
Leaves/Hibush	Mi-1	NpIII	Resistance against <i>Meloidogyne incognita</i>	First report of transgenic nematode-resistant eggplant	Frijters et al. (2000)
Cotyledon	luc	NpIII		Gene expression changes during a one-year period	Hanyu et al. (1999)
Root explants/MEBH 11, MEBH 9, Kalpatru and Rohini		NpIII		Optimization of factors for transformation efficiency	Maglioli et al. (2000)
Leaf explants/Haritha		NpIII		Variety-independent method for producing transgenic eggplant	Franklin and Sita (2003)
Cotyledon, hypocotyl and leaf explants/Pusa Purple Long		NpIII		Optimization of transformation protocol	Kannapiran (2003)
Leaf explants/Haritha		Hpt		Higher eggplant transformation efficiency using acetosyringone	Kumar and Rajam (2005)
Cotyledons/12 different cultivars	Bt (codon-optimized Cry2Aa)	NpIII	Resistance against brinjal shoot and fruit borer	Codon-optimized cry2Aa gene was used to obtain fully resistant plants	Soniya et al. (2005) Gupta et al. (2006)
HP83 and Moneymaker	Mi-1.2	NpIII	Resistance against root-knot nematodes (<i>Meloidogyne</i> spp.)	Used Mi-1.2 gene from tomato for resistance against nematodes but no effect on aphids	Goggin et al. (2006)
Hypocotyl segments/Embu		NpIII	Resistance against tomato chlorotic spot virus	Suggested that resistant phenotype depends on transgene copies	Piccoli et al. (2006)
Cotyledons/proprietary brinjal line	Bt (Cry2Ab)	NpIII	Resistance against fruit and shoot borer (<i>Leucinodes orbonalis</i>)	Simultaneously used T-DNA with cry2Ab gene and another T-DNA with nptII and GUS	Narendran (2006)
Hypocotyls/line IVBL-9	Bt (Cry1Ac)	NpIII	Resistance against shoot and fruit borer	Transformation frequency of 17.3% and 2.9 shoots per hypocotyl	Pal et al. (2009)
Green stem segments		aadA	Resistance against <i>Meloidogyne incognita</i>	Method for plastid transformation in eggplant	Singh et al. (2010)
Cotyledonary leaves/Co2	Bt (Cry1Ab)	NpIII	Resistance against Cucurbit Mosaic Virus (CMV)	Regeneration and transformation efficiencies were compared	Phap et al. (2010)
Cotyledonary leaves/Pusa Purple Long	CMV-CP	NpIII	Resistance against Cucurbit Mosaic Virus (CMV)	Resistance against cucumber mosaic virus	Pratap et al. (2011)
Hypocotyl/Kashi Taru	Synthetic Cry1Ac3	NpIII	Resistance against shoot and fruit borer (<i>Leucinodes orbonalis</i> Guenee)	Used a gene with full codon-modification	Rai et al. (2013)
Cotyledonary node/Jayant	Bt (Cry1Ac)	NpIII	Resistance against lepidopteron insects	Efficient in vitro regeneration protocol was developed	Kaur et al. (2013a)
Cotyledons/Ichiban	pEKH-WD	Ipt	Resistance against <i>Alternaria solani</i>	R/S site-specific recombination system	Darwish et al. (2014)
Cotyledon and hypocotyl	Bt (Cry1Ac)	Hpt		Successfully transformed cotyledons with Cry 1AC	Sidhu et al. (2014a)

(continued on next page)

Table 2 (continued)

Explant (tissue/genotype)	Gene	Selectable marker gene	Trait	Remarks	Reference
Cotyledon and leaf/Pusa Purple Long	<i>Chi</i>	<i>Hpt</i>	Resistance against fungal wilts	Higher chitinase activity	Singh et al. (2015)
Hypocotyl/Manjarigota		<i>NptII</i>		Standardization of shoot transformation efficiency	Prakash et al. (2015)
Shoot tip/Manjarigota, Ruchira, Poona selection, and Krishna Kathi	<i>Bt (Cry1F)</i>	<i>NptII</i>	Resistance against shoot and fruit borer (<i>Leucinodes orbonalis</i> Guenée)	Standardization for transformation efficiency	Jadhav et al. (2015)
Leaf disks/Pusa Purple Long	<i>OC-1dD86</i>	<i>NptII</i>	Resistance against root-knot nematodes (RKN)	Above 78% inhibition of root-knot nematode	Papolu et al. (2016)
Hypocotyl and Cotyledonary leaves/Manjarigota		<i>NptII</i>		Effects of growth regulators and explant-type on transformation method were studied	Prakash et al. (2016b)
Hypocotyl/Manjarigota		<i>NptII</i>		Effect of antibiotics and gelling agents was studied	Prakash et al. (2016a)
Hypocotyl/Arka Keshav and Manjarigota		<i>NptII</i>		Optimized the transformation system	Hanur et al. (2016)
Cotyledon and hypocotyls/RHRB-35 and Manjarigota	<i>Bt (Cry1Aabc)</i>	<i>NptII</i>	Resistance against shoot and fruit borer (<i>Leucinodes orbonalis</i> Guenée)	Factors influencing transformation efficiency were studied	Habde et al. (2017)
Fully expanded leaves/Pant Ritura		<i>NptII</i>		Transient expression in leaves	Kumar et al. (2017)
The nodal region of seedlings/PLR1		<i>NptII</i>		Standardization for gus gene expression	Jayabalan (2018)
<i>Abiotic stress tolerance</i>					
Leaf/Pusa Purple Long	<i>mtlD</i>	<i>NptII</i>	Tolerance against osmotic stress induced by salt, drought and chilling stress	First report of genetically engineered abiotic tolerance in eggplant	Prabhavathi et al. (2002)
Leaf and cotyledon explants/Pusa Purple Long	<i>adc</i>	<i>Hpt</i>	Stress tolerance	Increased tolerance levels to multiple abiotic stresses	Prabhavathi and Rajam (2007)
Shoot-tip/Utkal Anushree	<i>rd29A::DREB1A</i>	<i>NptII</i>	Tolerance to moisture stress	Transformation frequencies were compared	Sagare and Mohanty (2012)
Hypocotyledonary Leaf/PKM1	<i>HAL1</i>	<i>NptII</i>	Salt tolerance	Salt tolerant lines	Kumar et al. (2014)
<i>Other useful studies</i>					
Leaf, cotyledon and hypocotyl/female parent of the hybrid Rimina	<i>iaaMand DefH9</i> promoter		Parthenocarpic fruit development	Fruit set under unfavourable conditions	Rotino et al. (1997)
Hypocotyls/pure lines E-8 and E-38	<i>Barnase</i> and <i>Cre</i> gene	<i>Bar</i> , <i>NptII</i>	Pollen fertility restoration	Use of <i>Cre/loxP</i> system for fertility restoration	Cao et al. (2010)
Leaf and cotyledon/DR2	amiRNA targeting <i>SmTAF10</i> and <i>SmTAF13</i>	<i>NptII</i>	Male sterility	Complete transgene containment in eggplant	Toppino et al. (2011)
Leaf segments	Antisense <i>SmePPO1</i>	<i>Hpt</i>	Reduction of enzymatic browning	Reduced browning	Padma (2012)

fruits, as eggplant is among the top ten vegetables rich in phenolic acids (Kaushik et al., 2017, 2015).

7. Genome editing

Genome editing, or genome engineering, includes a set of techniques that allow to edit, delete, replace or insert, in a targeted site, specific genomic sequences of interest in the genome of a living organism (Gaj et al., 2016). Unlike genetic transformation techniques that randomly insert a gene into a host genome, genome editing targets the insertions to site-specific locations. Availability of sequenced genomes and the emergence of highly efficient editing technologies based on complexes that guide endonucleases allowed scientists to alter the plant genomes in a site-specific manner (Komor et al., 2017). These technologies are based on the induction of cuts in double-strand DNA (DSB, double-strand breaks), in genomes of different organisms including plants, which are then repaired either with the non-homologous end-joining (NHEJ) or with homology-directed repair (HDR) (Rinaldo and Ayliffe, 2015). There are four nuclease systems which can be used for inducing the DSBs (i) meganucleases, (ii) zinc finger nucleases (ZFN), (iii) transcription activator-like effector nucleases (TALEN) and, (iv) Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated nucleases (CRISPR/Cas). ZFN, TALEN and CRISPR/Cas are more efficient to 'edit' the 'target' sequences compared to meganucleases (Fig. 3). Recently, two excellent reviews presenting the importance of genome editing in vegetables especially in solanaceous food crops have been published (Cardi et al., 2017; Van Eck, 2018). The schematic representation of genome editing techniques is provided in Fig. 3.

Efficacy of CRISPR/Cas9 has been tested in tomato successfully (Brooks et al., 2014), targeting the neighbouring sequences in the second exon of the tomato homolog of Arabidopsis ARGONAUTE7 (SLAGO7) using CRISPR/Cas9 constructs and obtained the mutants having distinctive phenotypes. In another study, using CRISPR/Cas9 system, (Lor et al., 2014) efficiently mutagenized the RIN gene of tomato, which encodes a MADS-box transcription factor regulating fruit ripening and confirmed the important role of RIN in ripening. In 2016,

Thomazella et al. (2016), generated mutants showing resistance against different pathogens, including *Pseudomonas syringae* pv. tomato and *Phytophthora capsici* and *Xanthomonas* spp. by producing small deletions in the SLDMR6-1 gene using CRISPR/Cas-9 system. Hilioti et al. (2016) developed ZFN-based technology for the *S. lycopersicum* seed system and induced mutations in LEAFY-COTYLEDON1-LIKE4 (L1L4) gene leading to phenotypic diversity including fruit organ. Day-length sensitivity in crops limits their geographical range of cultivation, if photoperiod response of a crop is modified then the crop can be grown over geographical regions. Soyk et al. (2017) used CRISPR/Cas9 system to induce the mutations in SELF-PRUNING 5G (SP5G) causing rapid flowering and enhancing the compact determinate growth habit of field tomatoes ultimately increasing the yield. CRISPR/Cas9-mediated knock-out of polyphenol oxidase genes in eggplant (*S. melongena* L.) has also been reported (Gianoglio et al., 2017).

8. Prospects of molecular farming in eggplant

Plant molecular farming has emerged as a novel, promising branch of plant biotechnology where plants are engineered to produce recombinant pharmaceutical and industrial proteins in large quantities instead of using conventional bio-production systems including yeast, bacteria, and cultured insect and animal cells (Daniell et al., 2001; Horn et al., 2004; Obembe et al., 2011). This unique system offers several advantages over conventional bio-production systems in terms of safety, scale, cost-effectiveness, and the ease of distribution and storage. In recent past, a number of plant systems such as Arabidopsis (Nykiforuk et al., 2006), rice (Chung et al., 2014), carrot (Rojas-Anaya et al., 2009), tomato (Hiwasa-Tanase et al., 2012), etc., have been utilized as recombinant bio-factories for the expression of various proteins including potential vaccines and pharmaceuticals, through employing various modifications of recombinant processes and utilizing the most suitable tools and strategies. Later, focuses came to transiently express virus-like particles (look-alikes of viruses but without carrying infectious genomic material), which can be a source of novel vaccines (Marsian and Lomonosoff, 2016). At present, there are a series of beautifully written reviews showing the importance of this technology,

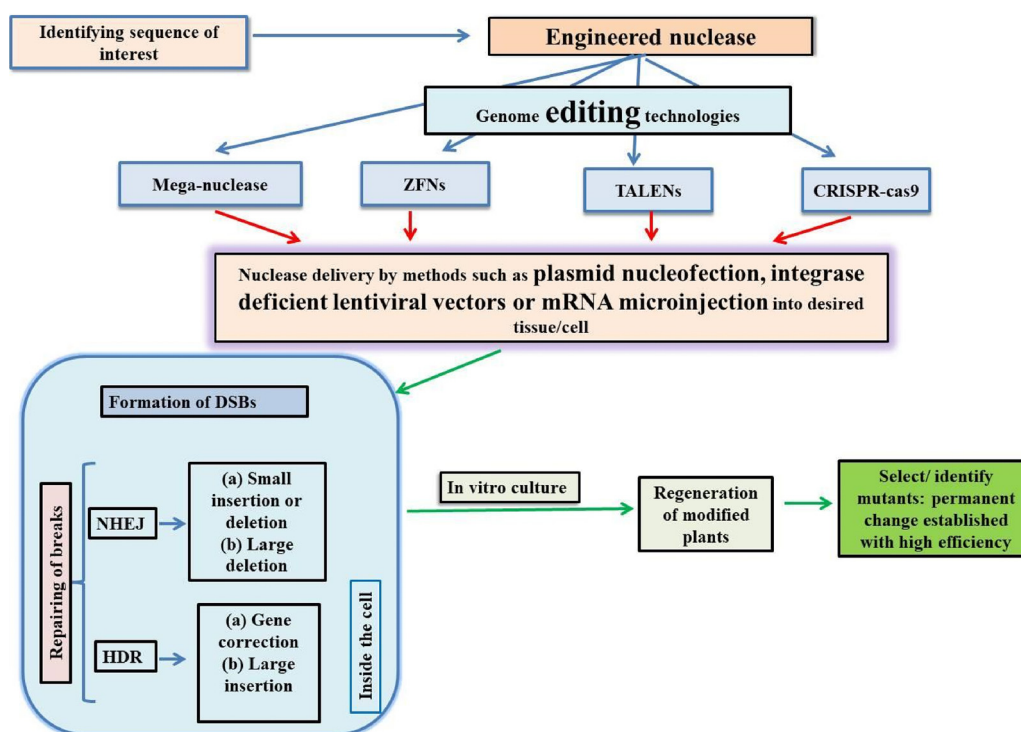


Fig. 3. Schematic representation for steps related to the delivery of an engineered nuclease for editing the genomes.



Fig. 4. Word cloud (bigger words means frequently used) based summary of tissue culture explants, and genes, markers, reporters, methods and traits.

advancements made in this field, and future prospects. But unfortunately, eggplant is lacking behind in this field and we could not find any report describing the use of eggplant in molecular farming. Although, eggplant possesses a huge underexploited diversity for fruit shape and size (Kaushik et al., 2016). Singh et al. (2010), developed a method for plastid transformation in eggplant. Development of this transplastomic technology in eggplant may provide exciting possibilities for novel gene introduction and expression in the engineered plastome for pharmaceutical traits in near future.

9. Concluding remarks

Plant biotechnology has played a crucial role in the improvement of vegetable crops. Plant biotechnology is a continuously evolving field and every day a new technology adds to the plant biotechnologist kit. Vegetables like tomato is a model for biotechnological studies. Eggplant genetic improvement is largely based on traditional plant breeding techniques. In the past decade eggplant breeding has made a significant improvement. Recently availability of molecular markers and the eggplant genome has helped plant breeder identify and incorporate better traits in the modern eggplant varieties. But, the development of biotechnological tools it lags the other members of Solanaceae.

Most of the transformation studies in eggplant are by agrobacterium mediated transformations, covering some biotic and abiotic challenges. There is an urgent need for eggplant varieties resistant against viral, fungal and bacterial pathogens along with a wider adaptability to climate change. Techniques like genome editing and molecular farming are yet to be explored in eggplant. Other methods for eggplant transformation have not been explored much. With a hiking trend in the

global population the sole development on traditional long plant breeding methods to end with a trait-based variety could be detrimental. Being a large-fruited vegetable eggplants open the prospects of molecular farming. Overall, summary is represented in Fig. 4.

References

- Alicchio, R., Del Grosso, E., Boschieri, E., 1982. Tissue cultures and plant regeneration from different explants in six cultivars of *Solanum melongena*. *Experientia* 38, 449–450.
- Alim, M.A., Biswas, B.K., Hasanuzzaman, M., Bala, P., Roy, S., 2015. Shoot regeneration and root induction in brinjal by growth regulators. *Res. Agric. Livestock Fish.* 2, 43–51.
- Ammirato, P.V., 1983. The regulation of somatic embryo development in plant cell cultures: suspension culture techniques and hormone requirements. *Nat. Biotechnol.* 1, 68.
- Aragão, F.J.L., Brasileiro, A.C.M., 2002. Positive, negative and marker-free strategies for transgenic plant selection. *Braz. J. Plant Physiol.* 14, 01–10. <https://doi.org/10.1590/S1677-04202002000100001>.
- Arpaia, S., Mennella, G., Onofaro, V., Perri, E., Sunseri, F., Rotino, G.L., 1997. Production of transgenic eggplant (*Solanum melongena* L.) resistant to Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Theoret. Appl. Genet.* 95, 329–334.
- Behera, T.K., Singh, N., 2002. Inter-specific crosses between eggplant (*Solanum melongena* L.) with related *Solanum* species. *Scientia Hort.* 95, 165–172.
- Bhat, S.V., Jadhav, A.S., Pawar, B.D., Kale, A.A., Chimote, V.P., Pawar, S.V., 2013. In vitro shoot organogenesis and plantlet regeneration in brinjal (*Solanum melongena* L.). *The Bioscan* 8, 821–823.
- Billings, S., Jelenkovic, G., Chin, C.-K., Eberhardt, J., 1997. The effect of growth regulators and antibiotics on eggplant transformation. *J. Am. Soc. Hortic. Sci.* 122, 158–162.
- Binns, A.N., 1983. Host and T-DNA determinants of cytokinin autonomy in tobacco cells transformed by *Agrobacterium tumefaciens*. *Planta* 158, 272–279. <https://doi.org/10.1007/BF01075264>.
- Bisognin, D.A., 2011. Breeding vegetatively propagated horticultural crops. *Crop Breed. Appl. Biotechnol.* 11, 35–43. <https://doi.org/10.1590/S1984-70332011000500006>.

- Borlaug, N.E., 1983. Contributions of conventional plant breeding to food production. *Science* 219, 689–693.
- Brooks, C., Nekrasov, V., Lippman, Z.B., Van Eck, J., 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. *Plant Physiol.* 166, 1292–1297. <https://doi.org/10.1104/pp.114.247577>.
- Cao, B., Huang, Z., Chen, G., Lei, J., 2010. Restoring pollen fertility in transgenic male-sterile eggplant by Cre/loxP-mediated site-specific recombination system. *Genet. Mol. Biol.* 33, 298–307.
- Cardi, T., D'Agostino, N., Tripodi, P., 2017. Genetic transformation and genomic resources for next-generation precise genome engineering in vegetable crops. *Front. Plant Sci.* 8, 241. <https://doi.org/10.3389/fpls.2017.00241>.
- Charaya, P., Sanghera, G.S., Gossal, S.S., 2011. Effect of genotype and explants on direct in vitro plant regeneration in brinjal (*Solanum melongena* L.). *J. Plant Sci. Res.* 27, 37.
- Chase, C.D., 2006. Genetically engineered cytoplasmic male sterility. *Trends Plant Sci.* 11, 7–9.
- Chen, Q., Jelenkovic, G., Chin, C.-K., Billings, S., Eherhardt, J., Goffreda, J.C., et al., 1995. Transfer and transcriptional expression of coleopteran CryIIIB endotoxin gene of *Bacillus thuringiensis* in eggplant. *J. Am. Soc. Hortic. Sci.* 120, 921–927.
- Chow, Y.T., Chen, S., Wang, R., Liu, C., Kong, C., Li, R.A., et al., 2016. Single cell transfection through precise microinjection with quantitatively controlled injection volumes. *Sci. Rep.* 6. <https://doi.org/10.1038/srep24127>.
- Christou, P., 1992. Genetic transformation of crop plants using microprojectile bombardment. *Plant J.* 2, 275–281.
- Chung, N.D., Kim, N.S., Giap, V. do, Jang, S.H., Oh, S.M., Jang, S.H., et al., 2014. Production of functional human vascular endothelial growth factor(165) in transgenic rice cell suspension cultures. *Enzyme Microb. Technol.* 63, 58–63. <https://doi.org/10.1016/j.enzmictec.2014.05.007>.
- Collonnier, C., Fock, I., Kashyap, V., Rotino, G.L., Daunay, M.C., Lian, Y., et al., 2001. Applications of biotechnology in eggplant. *Plant Cell Tissue Organ Culture* 65, 91–107.
- Dalal, M., Dani, R.G., Kumar, P.A., 2006. Current trends in the genetic engineering of vegetable crops. *Scientia Hortic.* 107, 215–225.
- Daniell, H., Streatfield, S.J., Wycoff, K., 2001. Medical molecular farming: production of antibodies, biopharmaceuticals and edible vaccines in plants. *Trends Plant Sci.* 6, 219–226.
- Darwish, N.A., Khan, R.S., Ntui, V.O., Nakamura, I., Mii, M., 2014. Generation of selectable marker-free transgenic eggplant resistant to *Alternaria solani* using the R/RS site-specific recombination system. *Plant Cell Rep.* 33, 411–421.
- Daunay, M.C., Hazra, P., 2012. Eggplant. *Handb. Vegetables* 257–322.
- Daunay, M.-C., 2008. Eggplant. *Vegetables II Handbook of Plant Breeding*. Springer, New York, NY, pp. 163–220. https://doi.org/10.1007/978-0-387-74110-9_5.
- Dennis, E.S., Ellis, J., Green, A., Llewellyn, D., Morell, M., Tabe, L., et al., 2008. Genetic contributions to agricultural sustainability. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 363, 591–609. <https://doi.org/10.1098/rstb.2007.2172>.
- FAOSTAT, 2016. FAO Statistical Database. (accessed 1.11.19).
- Farhad, T., Soleiman, J., Fahrul, H., 2015. High performant eggplant in vitro regeneration and organogenesis. *Agroecol. J.* 11, 24–29.
- Fári, M., Nagy, I., Csányi, M., Mitykó, J., Andrásfalvy, A., 1995. Agrobacterium mediated genetic transformation and plant regeneration via organogenesis and somatic embryogenesis from cotyledon leaves in eggplant (*Solanum melongena* L. cv. 'Kecskeméti lila'). *Plant Cell Rep.* 15, 82–86.
- Fassuliotis, G., Nelson, B.V., Bhatt, D.P., 1981. Organogenesis in tissue culture of *Solanum melongena* cv. Florida Market. *Plant Sci. Lett.* 22, 119–125.
- Ferdousi, A., Nath, U.K., Das, B.L., Alam, M.S., 2009. In vitro regeneration system in brinjal (*Solanum melongena* L.) for stress tolerant somaclonal selection. *J. Bangladesh Agric. Univ.* 7, 253–258.
- Filippone, E., Lurquin, P.F., 1989. Stable transformation of eggplant (*Solanum melongena* L.) by cocultivation of tissues with *Agrobacterium tumefaciens* carrying a binary plasmid vector. *Plant Cell Rep.* 8, 370–373.
- Fobert, P.R., Webb, D.T., 1988. Effects of polyamines, polyamine precursors, and polyamine biosynthetic inhibitors on somatic embryogenesis from eggplant (*Solanum melongena*) cotyledons. *Can. J. Bot.* 66, 1734–1742.
- Foo, P.C., Lee, Z.H., Chin, C.K., Subramaniam, S., Chew, B.L., 2018. Shoot induction in white eggplant (*Solanum melongena* L. Cv. Bulat Putih) using 6-benzylaminopurine and kinetin. *Trop. Life Sci. Res.* 29, 119.
- Franklin, G., Lakshmi Sita, G., 2003. Agrobacterium *tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L.) using root explants. *Plant Cell Rep.* 21, 549–554. <https://doi.org/10.1007/s00299-002-0546-9>.
- Franklin, G., Sheeba, C.J., Sita, G.L., 2004. Regeneration of eggplant (*Solanum melongena* L.) from root explants. *In Vitro Cell. Dev. Biol.-Plant* 40, 188–191.
- Franklin, G., Sita, G.L., 2003. Agrobacterium *tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L.) using root explants. *Plant Cell Rep.* 21, 549–554.
- Frijters, A., Simons, G., Varga, G., Quaedvlieg, N., de Both, M., 2000. The Mi-1 gene confers resistance to the root-knot nematode *Meloidogyne incognita* in transgenic eggplant. VIIIth Conf. 'Plant and Animal Genome', San Diego, CA, USA.
- Gaj, T., Sirk, S.J., Shui, S., Liu, J., 2016. Genome-editing technologies: principles and applications. *Cold Spring Harb. Perspect. Biol.* 8, a023754. <https://doi.org/10.1101/cshperspect.a023754>.
- Gelvin, S.B., 2003. Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiol. Mol. Biol. Rev.* 67, 16–37. <https://doi.org/10.1128/MMBR.67.1.16-37.2003>.
- Gianoglio, S., Comino, C., Moglia, A., Lanteri, S., Jaime, P., Diego, O., et al., 2017. CRISPR/Cas9-mediated Knock-out of Polyphenol Oxidase Genes in Eggplant (*Solanum melongena* L.). UPV Universitat Politècnica de València. Available at: <https://iris.univ.it/handle/2318/1652307#XEt6yB7nD4> [Accessed January 25, 2019], pp. 159–159.
- Gisbert, C., Prohens, J., Nuez, F., 2006. Efficient regeneration in two potential new crops for subtropical climates, the scarlet (*Solanum aethiopicum*) and gboma (*S. macrocarpon*) eggplants. *N. Zeal. J. Crop Hortic. Sci.* 34, 55–62.
- Gledde, S., Keller, W., Setterfield, G., 1983. Somatic embryogenesis and plant regeneration from leaf explants and cell suspensions of *Solanum melongena* (eggplant). *Can. J. Bot.* 61, 656–666.
- Goggin, F.L., Jia, L., Shah, G., Hebert, S., Williamson, V.M., Ullman, D.E., 2006. Heterologous expression of the Mi-1.2 gene from tomato confers resistance against nematodes but not aphids in eggplant. *Mol. Plant-Microbe Interact.* 19, 383–388.
- Gramazio, P., 2018. Genetics and Genomics of Cultivated Eggplants and Wild Relatives. <https://doi.org/10.4995/Thesis/10251/104605>.
- Grimsley, N., Hohn, B., Hohn, T., Walden, R., 1986. "Agroinfection" an alternative route for viral infection of plants by using the Ti plasmid. *Proc. Natl. Acad. Sci.* 83, 3282–3286.
- Guang-yuan, W.F.L., Sha-sha, P.A.N., 2007. In vitro culture of cotyledons and under hypocotyls of eggplants [J]. *J. Jilin Agric. Univ.* 4, 012.
- Gupta, R., Veilleux, R.E., Welbaum, G.E., 2006. PCR based synthesis of codon optimized cry2Aa gene for production of fruit and shoot borer (*Leucinodes orbonalis*) resistant eggplant (*Solanum melongena* L.) cultivars. *Korean Soc. Hortic. pp.* 359–359.
- Guri, A., Sink, K.C., 1988. Agrobacterium transformation of eggplant. *J. Plant Physiol.* 133, 52–55.
- Habde, S., Jadhav, A., Kulwal, P., Pawar, B., 2017. Development of fruit and shoot borer resistant transgenic brinjal with CryIAbc gene: an assessment of factors influencing transformation efficiency. *J. Pharm. Phytochem.* 6, 2009–2013.
- Habib, M.A., Mukhtadir, M.A., Kabir, M.R., Mian, M.K., Akhond, M.Y., 2016. Optimization of somatic embryogenesis protocol for locally adapted eggplant (*Solanum melongena* L.) varieties in Bangladesh. *Plant Tissue Culture Biotechnol.* 26, 77–83.
- Hamilton, G.C., Jelenkovic, G.L., Lashomb, J.H., Ghidui, G., Billings, S., Patt, J.M., 1997. Effectiveness of transgenic eggplant (*Solanum melongena* L.) against the Colorado potato beetle. *Adv. Hortic. Sci.* 189–192.
- Han, L., Zhang, L., Liu, J., Li, H., Wang, Y., Hasi, A., 2015. Transient expression of optimized and synthesized nattokinase gene in melon (*Cucumis melo* L.) fruit by agroinfiltration. *Plant Biotechnol.* 15.0430a. <https://doi.org/10.5511/plantbiotechnology.15.0430a>.
- Hanur, V.S., Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Mahmood, R., et al., 2016. Synergistic use of hypocotyl explants and high BAP preconditioning for enhanced transformation frequency in brinjal (*Solanum melongena* L.). *J. Hortic. Sci.* 1, 116–119.
- Hanyu, H., Murata, A., Park, E.Y., Okabe, M., Billings, S., Jelenkovic, G., et al., 1999. Stability of luciferase gene expression in a long term period in transgenic eggplant, *Solanum melongena*. *Plant Biotechnol.* 16, 403–407.
- Hare, P.D., Chua, N.-H., 2002. Excision of selectable marker genes from transgenic plants. *Nat. Biotechnol.* 20, 575.
- Hilioti, Z., Ganopoulos, I., Ajith, S., Bossis, I., Tsafaris, A., 2016. A novel arrangement of zinc finger nuclease system for in vivo targeted genome engineering: the tomato LEC1-LIKE4 gene case. *Plant Cell Rep.* 35, 2241–2255. <https://doi.org/10.1007/s00299-016-2031-x>.
- Hiwasa-Tanase, K., Hirai, T., Kato, K., Duhita, N., Ezura, H., 2012. From miracle fruit to transgenic tomato: mass production of the taste-modifying protein miraculin in transgenic plants. *Plant Cell Rep.* 31, 513–525. <https://doi.org/10.1007/s00299-011-1197-5>.
- Horn, M.E., Woodard, S.L., Howard, J.A., 2004. Plant molecular farming: systems and products. *Plant Cell Rep.* 22, 711–720.
- Horstman, A., Bemer, M., Boutilier, K., 2017. A transcriptional view on somatic embryogenesis. *Regeneration (Oxf.)* 4, 201–216. <https://doi.org/10.1002/reg.291>.
- Huda, A., Bari, M.A., Rahman, M., Nahar, N., 2007. Somatic embryogenesis in two varieties of eggplant (*Solanum melongena* L.). *Res. J. Bot.* 2, 195–201.
- Jacobsen, E., Schouten, H.J., 2007. Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends Biotechnol.* 25, 219–223. <https://doi.org/10.1016/j.tibtech.2007.03.008>.
- Jadhav, M., Jadhav, A., Pawar, B., Kale, A., Kute, N., 2015. Agrobacterium-mediated genetic transformation of brinjal with cry1F gene for resistance against shoot and fruit borer. *J. Crop Improve.* 29, 518–527.
- Jailani, A.A.K., Kumar, A., Mandal, B., Sivasudha, T., Roy, A., 2016. Agroinfection of tobacco by croton yellow vein mosaic virus and designing of a replicon vector for expression of foreign gene in plant. *Virus Disease* 27, 277–286.
- Jayabalan, N., 2018. Transformation and confirmation of GUS gene expression in *Solanum melongena* L. of PLR 1 cultivar. *Annals Plant Sci.* 7, 2185–2193.
- Jelenkovic, G., Billings, S., Chen, Q., Lashomb, J., Hamilton, G., Ghidui, G., 1998. Transformation of eggplant with synthetic cryIIIA gene produces a high level of resistance to the Colorado potato beetle. *J. Am. Soc. Hortic. Sci.* 123, 19–25.
- Kamat, M.G., Rao, P.S., 1978. Vegetative multiplication of eggplants (*Solanum melongena*) using tissue culture techniques. *Plant Sci. Lett.* 13, 57–65.
- Kanna, S.V., Jayabalan, N., 2010. Influence of n6-(2-isopentenyl) adenine on in vitro shoot proliferation in *Solanum melongena* L. *Int. J. Acad. Res.* 2, 98.
- Kanna, S.V., Jayabalan, N., 2015. Regeneration via direct organogenesis from leaf segments of eggplant (*Solanum melongena* L.). *J. Plant Sci.* 10, 90–98.
- Kannapiran, P., 2003. Genetic transformation in *Solanum melongena* L. thesis. <http://krishikosh.egranth.ac.in/handle/1/64706>.
- Kantharajah, A.S., Golegaonkar, P.G., 2004. Somatic embryogenesis in eggplant. *Scientia Hortic.* 99, 107–117.
- Kashyap, V., Kumar, S.V., Collonnier, C., Fusari, F., Haicour, R., Rotino, G.L., et al., 2003. Biotechnology of eggplant. *Scientia Hortic.* 97, 1–25.
- Kaur, M., Dhath, A.S., Sandhu, J.S., Gosal, S.S., 2015. Effect of genotype, explant and culture media on direct plant regeneration in eggplant. *Hortic. Soc. India (Regd.)* 72,

- 232–238.
- Kaur, M., Dhath, A.S., Sandhu, J.S., Gosal, S.S., Sidhu, G.S., 2013a. Agrobacterium transformation of eggplant (*Solanum melongena* L.) for glucuronidase expression. *J. Cell Tissue Res.* 13.
- Kaur, M., Dhath, A.S., Sandhu, J.S., Sidhu, A.S., Gosal, S.S., 2013b. Effect of media composition and explant type on the regeneration of eggplant (*Solanum melongena* L.). *Afr. J. Biotechnol.* 12.
- Kaur, M., Singh Dhath, A., Singh Sandhu, J., Singh Sidhu, A., Singh Gosal, S., 2011. Role of genotype, explant and growth hormones on regeneration in eggplant (*Solanum melongena*). *Indian J. Agric. Sci.* 81, 38.
- Kaushik, P., Andújar, I., Vilanova, S., Plazas, M., Gramazio, P., Herraiz, F.J., Brar, N.S., Prohens, J., 2015. Breeding vegetables with increased content in bioactive phenolic acids. *Molecules* 20, 18464–18481. <https://doi.org/10.3390/molecules201018464>.
- Kaushik, P., Gramazio, P., Vilanova, S., Raigón, M.D., Prohens, J., Plazas, M., 2017. Phenolics content, fruit flesh colour and browning in cultivated eggplant, wild relatives and interspecific hybrids and implications for fruit quality breeding. *Food Res. Int.* 102, 392–401. <https://doi.org/10.1016/j.foodres.2017.09.028>.
- Kaushik, P., Prohens, J., Vilanova, S., Gramazio, P., Plazas, M., 2016. Phenotyping of eggplant wild relatives and interspecific hybrids with conventional and phenomics descriptors provides insight for their potential utilization in breeding. *Front. Plant Sci.* 7, 677. <https://doi.org/10.3389/fpls.2016.00677>.
- Kole, C., Michler, C., Abbott, A.G., Hall, T.C., 2010. Generation and deployment of transgenic crop plants: an overview. *Transgenic Crop Plants: Volume 1: Principles and Development*. Springer Science & Business Media, pp. 1–29.
- Komari, T., 1989. Transformation of callus cultures of nine plant species mediated by Agrobacterium. *Plant Sci.* 60, 223–229.
- Komor, A.C., Badran, A.H., Liu, D.R., 2017. CRISPR-based technologies for the manipulation of eukaryotic genomes. *Cell* 168, 20–36. <https://doi.org/10.1016/j.cell.2016.10.044>.
- Kouassi, B., Prohens, J., Gramazio, P., Kouassi, A.B., Vilanova, S., Galán-Ávila, A., et al., 2016. Development of backcross generations and new interspecific hybrid combinations for introgression breeding in eggplant (*Solanum melongena*). *Scientia Hort.* 213, 199–207. <https://doi.org/10.1016/j.scienta.2016.10.039>.
- Kumar, P.A., Mandaokar, A., Sreenivasu, K., Chakrabarti, S.K., Bisaria, S., Sharma, S.R., et al., 1998. Insect-resistant transgenic brinjal plants. *Mol. Breed.* 4, 33–37.
- Kumar, S.K., Sivanesan, I., Murugesan, K., Jeong, B.R., Hwang, S.J., 2014. Enhancing salt tolerance in eggplant by introduction of foreign halotolerance gene, HAL1 isolated from yeast. *Hortic. Environ. Biotechnol.* 55, 222–229.
- Kumar, S.V., Rajam, M.V., 2005. Enhanced induction of vir genes results in the improvement of Agrobacterium-mediated transformation of eggplant. *J. Plant Biochem. Biotechnol.* 14, 89–94.
- Kumar, V., Kumar, P., Chattopadhyay, T., 2017. A simple method for transient expression of reporter gene in brinjal leaves through agroinfiltration. *Int. J. Curr. Microbiol. Appl. Sci.* 6, 317–323.
- Lee, M.W., Yang, Y., 2006. Transient expression assay by agroinfiltration of leaves. *Arabidopsis Protocols Methods in Molecular Biology*. Humana Press, pp. 225–229. <https://doi.org/10.1385/1-59745-003-0:225>.
- Lei, S.A., 2010. Benefits and costs of vegetative and sexual reproduction in perennial plants: a review of literature. *J. Arizona-Nevada Acad. Sci.* 42, 9–14.
- Leuzinger, K., Dent, M., Hurtado, J., Stahnke, J., Lai, H., Zhou, X., et al., 2013. Efficient agroinfiltration of plants for high-level transient expression of recombinant proteins. *J. Vis. Exp.* <https://doi.org/10.3791/50521>.
- LianYong, L.F., Yuhui, C., Yan, S., Songlin, Z., Sihachakr, D., 2004. Plantlet regeneration by isolated microspore culture of somatic hybrid of eggplant [J]. *Acta Hort.* Sin. 2, 024.
- Lin, J.-Y., Chiou, T.-J., Lo, H.-F., Lin, S.-I., 2016. Development of an in planta system to monitor phosphorus status by agroinfiltration and agroinjection. *Plant Soil* 409, 313–328.
- Litz, R.E., 1993. Organogenesis and somatic embryogenesis. *Acta Hort.* 199–206. <https://doi.org/10.17660/ActaHortic.1993.336.25>.
- Lor, V.S., Starker, C.G., Voytas, D.F., Weiss, D., Olszewski, N.E., 2014. Targeted mutagenesis of the tomato PROCERA gene using transcription activator-like effector nucleases. *Plant Physiol.* 166, 1288–1291. <https://doi.org/10.1104/pp.114.247593>.
- Luft, C., Ketteler, R., 2015. Electroporation knows no boundaries. *J. Biomol. Screen.* 20, 932–942. <https://doi.org/10.1177/1087057115579638>.
- Lusser, M., Parisi, C., Plan, D., Rodríguez-Cerezo, E., 2012. Deployment of new biotechnologies in plant breeding. *Nat. Biotechnol.* 30, 231.
- Lynch, P.T., 2014. Tissue culture techniques in in vitro plant conservation. *Plant Conservation Biotechnology*. CRC Press, pp. 67–88.
- Maghari, B.M., Ardekani, A.M., 2011. Genetically modified foods and social concerns. *Avicenna J. Med. Biotechnol.* 3, 109–117.
- Magioli, C., Mansur, E., 2005. Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant. *Acta Bot. Bras.* 19, 139–148. <https://doi.org/10.1590/S0102-33062005000100013>.
- Magioli, C., da Rocha, A.P.M., Pinheiro, M.-M., Martins, G.-S., Mansur, E., 2000. Establishment of an efficient Agrobacterium transformation system for eggplant and study of a potential biotechnologically useful promoter. *J. Plant Biotechnol.* 2, 43–49.
- Magioli, C., Rocha, A.P.M., De Oliveira, D.E., Mansur, E., 1998. Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. *Plant Cell Rep.* 17, 661–663.
- Mallaya, N.P., Ravishankar, G.A., 2013. In vitro propagation and genetic fidelity study of plant regenerated from inverted hypocotyl explants of eggplant (*Solanum melongena* L.) cv. Arka Shirish. *Biotech* 3, 45–52. <https://doi.org/10.1007/s13205-012-0068-2>.
- Marco, F., Bitrián, M., Carrasco, P., Rajam, M.V., Alcázar, R., Tiburcio, A.F., 2015. Genetic engineering strategies for abiotic stress tolerance in plants. *Plant Biology and Biotechnology*. Springer, New Delhi, pp. 579–609. https://doi.org/10.1007/978-81-322-2283-5_29.
- Marsian, J., Lomonossoff, G.P., 2016. Molecular pharming—VLPs made in plants. *Curr. Opin. Biotechnol.* 37, 201–206.
- Matsuoka, H., Hinata, K., 1979. NAA-induced organogenesis and embryogenesis in hypocotyl callus of *Solanum melongena* L. *J. Exp. Bot.* 30, 363–370.
- Mba, C., Guimaraes, E.P., Ghosh, K., 2012. Re-orienting crop improvement for the changing climatic conditions of the 21st century. *Agric. Food Security* 1, 7.
- Miki, B., McHugh, S., 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. *J. Biotechnol.* 107, 193–232.
- Mir, K.A., Dhath, A.S., Sandhu, J.S., Gosal, S.S., 2008. Genotype, explant and culture medium effects on somatic embryogenesis in eggplant (*Solanum melongena* L.). *Hortic. Environ. Biotechnol.* 49, 182–187.
- Mir, K.A., Dhath, A.S., Sandhu, J.S., Sidhu, A.S., 2011. Effect of genotype, explant and culture medium on organogenesis in brinjal. *Indian J. Hort.* 68, 332–335.
- Moradpour, M., Abdullah, S.N.A., 2017. Cisgenesis and intragenesis as new strategies for crop improvement. *Crop Improvement*. Springer, Cham, pp. 191–216. https://doi.org/10.1007/978-3-319-65079-1_9.
- Mukherjee, S.K., Rathinasabapathi, B., Gupta, N., 1991. Low sugar and osmotic requirements for shoot regeneration from leaf pieces of *Solanum melongena* L. *Plant Cell Tissue Organ Culture* 25, 13–16.
- Muktadir, M.A., Habib, M.A., Mian, M.A.K., Akhond, M.A.Y., 2016. Regeneration efficiency based on genotype, culture condition and growth regulators of eggplant (*Solanum melongena* L.). *Agric. Natural Resour.* 50, 38–42.
- Muthusamy, A., Vidya, K.S., Pratibha, P.K., Rao, M.R., Vidhu, S.B., Guruprasad, K.P., et al., 2014. Establishment of an in vitro plantlet regeneration protocol for unique varieties of brinjal (*Solanum melongena* L.) var. Mattu Gulla and Perampalli Gulla. *Indian J. Exp. Biol.* 52, 80–88.
- Narendran, M., 2006. Production of marker-free insect resistant transgenic brinjal (*Solanum melongena* L.) carrying cry2Ab gene. *International Conference on Indigenous Vegetables and Legumes. Prospectus for Fighting Poverty, Hunger and Malnutrition*, 752 535–538.
- Natarajan, S., Turna, J., 2007. Excision of selectable marker genes from transgenic crops as a concern for environmental biosafety. *J. Sci. Food Agric.* 87, 2547–2554. <https://doi.org/10.1002/jsfa.2831>.
- Nester, E.W., 2015. Agrobacterium: nature's genetic engineer. *Front. Plant Sci.* 5, 1–16. <https://doi.org/10.3389/fpls.2014.00730>.
- Norkunas, K., Harding, R., Dale, J., Dugdale, B., 2018. Improving agroinfiltration-based transient gene expression in *Nicotiana benthamiana*. *Plant Methods* 14, 71.
- Nyaboga, E., Tripathi, J.N., Manoharan, R., Tripathi, L., 2014. Agrobacterium-mediated genetic transformation of yam (*Dioscorea rotundata*): an important tool for functional study of genes and crop improvement. *Front. Plant Sci.* 5. <https://doi.org/10.3389/fpls.2014.00463>.
- Nykiforuk, C.L., Boothe, J.G., Murray, E.W., Keon, R.G., Goren, H.J., Markley, N.A., et al., 2006. Transgenic expression and recovery of biologically active recombinant human insulin from *Arabidopsis thaliana* seeds. *Plant Biotechnol. J.* 4, 77–85.
- Obembe, O.O., Popoola, J.O., Leelavathi, S., Reddy, S.V., 2011. Advances in plant molecular farming. *Biotechnol. Adv.* 29, 210–222.
- Padma, N.M., 2012. In vitro regeneration of *Solanum melongena* L. and genetic transformation towards reduction of enzymatic browning. *Afr. J. Biotechnol.* 13, 714–722.
- Pal, J.K., Singh, M., Rai, M., Satpathy, S., Singh, D.V., Kumar, S., 2009. Development and bioassay of Cry1Ac-transgenic eggplant (*Solanum melongena* L.) resistant to shoot and fruit borer. *J. Hort.* Sci. Biotechnol. 84, 434–438.
- Papoli, P.K., Dutta, T.K., Tyagi, N., Urwin, P.E., Lilley, C.J., Rao, U., 2016. Expression of a cystatin transgene in eggplant provides resistance to root-knot nematode, *Meloidogyne incognita*. *Front. Plant Sci.* 7, 1122.
- Phap, P.D., Xuan, H.T.L., Sudhakar, D., Balasubramanian, P., 2010. Engineering resistance in brinjal against nematode (*Meloidogyne incognita*) using cry1Ab gene from *Bacillus thuringiensis* Berliner. *The Third International Conference on the Development of Biomedical Engineering in Vietnam* (Springer) 278–281.
- Picoli, E.A.T., Lima, G.S.A., Lau, D., Oliveira, J.C.F., Laia, M.L., Zerbini, F.M., et al., 2006. Resistance gene Sw-5 of tomato confers resistance to TCSV in *Solanum melongena*. *Int. J. Hort.* Sci. 12, 41–47.
- Picoli, E.D.T., Cecon, P.R., Fari, M.G., Otoni, W.C., 2002. Organogenesis in eggplant (*Solanum melongena* L. cv. Embú) as affected by antibiotics and growth regulators. *Int. J. Hort.* Sci. 8, 76–82.
- Plazas, M., Vilanova, S., Gramazio, P., Rodríguez-Burruezo, A., Fita, A., Herraiz, F.J., et al., 2016. Interspecific hybridization between eggplant and wild relatives from different gene pools. *J. Am. Soc. Hort.* Sci. 141, 34–44.
- Potrykus, I., Paszkowski, J., Saul, M.W., Petruska, J., Shillito, R.D., 1985. Molecular and general genetics of a hybrid foreign gene introduced into tobacco by direct gene transfer. *Mol. Gen. Genet.* 199, 169–177. <https://doi.org/10.1007/BF00330255>.
- Prabhavathi, V.R., Rajam, M.V., 2007. Polyamine accumulation in transgenic eggplant enhances tolerance to multiple abiotic stresses and fungal resistance. *Plant Biotechnol.* 24, 273–282.
- Prabhavathi, V., Yadav, J.S., Kumar, P.A., Rajam, M.V., 2002. Abiotic stress tolerance in transgenic eggplant (*Solanum melongena* L.) by introduction of bacterial mannitol phosphohydrogenase gene. *Mol. Breed.* 9, 137–147.
- Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Anand, L., Hanur, V.S., 2016a. Effect of antibiotics and gelling agents in transformation of brinjal (*Solanum melongena* L.) cv. Manjarigota. *J. Hort.* Sci. 2, 19–25.
- Prakash, D.P., Deepali, B.S., Asokan, R., Ramachandra, Y.L., Anand, L., Hanur, V.S., 2016b. Effects of growth regulators and explant-type on agrobacterium-mediated transformation in brinjal (*Solanum melongena* L.) cv. Manjarigota. *J. Hort.* Sci. 2, 94–98.
- Prakash, D.P., Ramachandra, Y.L., Hanur, V.S., 2015. Factors affecting in vitro shoot regeneration in hypocotyls of brinjal (*Solanum melongena* L.) in the early steps of

- Agrobacterium-mediated transformation. *J. Hortic. Sci.* 10, 136–142.
- Pratap, D., Kumar, S., Raj, S.K., Sharma, A.K., 2011. Agrobacterium-mediated transformation of eggplant (*Solanum melongena* L.) using cotyledon explants and coat protein gene of Cucumber mosaic virus. *Indian J. Biotechnol.* 10, 19–24.
- Puchta, H., Fausser, F., 2013. Gene targeting in plants: 25 years later. *Int. J. Dev. Biol.* 57, 629–637.
- Rahman, M., Asaduzzaman, M., Nahar, N., Bari, M.A., 2006. Efficient plant regeneration from cotyledon and midrib derived callus in eggplant (*Solanum melongena* L.). *J. Bio-Sci.* 14, 31–38.
- Rai, N.P., Rai, G.K., Kumar, S., Kumari, N., Singh, M., 2013. Shoot and fruit borer resistant transgenic eggplant (*Solanum melongena* L.) expressing cry1Aa3 gene: development and bioassay. *Crop Protection* 53, 37–45.
- Ranil, R.H.G., Prohens, J., Aubriot, X., Niran, H.M.L., Plazas, M., Fonseka, R.M., et al., 2017. *Solanum insaanum* L. (subgenus *Leptostemonum* Bitter, Solanaceae), the neglected wild progenitor of eggplant (*S. melongena* L.): a review of taxonomy, characteristics and uses aimed at its enhancement for improved eggplant breeding. *Genet. Resour. Crop Evol.* 64, 1707–1722. <https://doi.org/10.1007/s10722-016-0467-z>.
- Rao, P.L., Singh, B., 1991. Plantlet regeneration from encapsulated somatic embryos of hybrid *Solanum melongena* L. *Plant Cell Rep.* 10, 7–11.
- Ray, B.P., Hassan, L., Sarker, S.K., 2010. Plant regeneration from seedling derived explants through callus of eggplant (*Solanum melongena* L.). *Agriculturists* 8, 98–107.
- Rinaldo, A.R., Ayliffe, M., 2015. Gene targeting and editing in crop plants: a new era of precision opportunities. *Mol. Breeding* 35, 40. <https://doi.org/10.1007/s11032-015-0210-z>.
- Rivas-Sendra, A., Corral-Martínez, P., Camacho-Fernández, C., Seguí-Simarro, J.M., 2015. Improved regeneration of eggplant doubled haploids from microspore-derived calli through organogenesis. *Plant Cell Tissue Organ Culture (PCTOC)* 122, 759–765.
- Robinson, J.P., Saranya, S., 2013. An improved method for the in vitro propagation of *Solanum melongena* L. *Int. J. Curr. Microbiol. Appl. Sci.* 2, 299–306.
- Rojas-Anaya, E., Loza-Rubio, E., Olivera-Flores, M.T., Gomez-Lim, M., 2009. Expression of rabies virus G protein in carrots (*Daucus carota*). *Transgenic Res.* 18, 911–919. <https://doi.org/10.1007/s11248-009-9278-8>.
- Rotino, G.L., Arpaia, S., Iannaccone, R., Iannamico, V., Mennella, G., Onofaro, V., et al., 1992. Agrobacterium-mediated transformation of *Solanum* spp. using a Bt gene effective against coleopterans. In: *Proc. VIIIth Meeting on Genetics and Breeding of Capsium and Eggplant*. Rome, Italy. pp. 7–10.
- Rotino, G.L., Gledie, S., 1990. Transformation of eggplant (*Solanum melongena* L.) using a binary *Agrobacterium tumefaciens* vector. *Plant Cell Rep.* 9, 26–29.
- Rotino, G.L., Perri, E., Acciarri, N., Sunseri, F., Arpaia, S., 1997. Development of eggplant varietal resistance to insects and diseases via plant breeding. *Adv. Hortic. Sci.* 193–201.
- Rotino, G.L., Sala, T., Toppino, L., 2014. Eggplant. *Alien Gene Transfer in Crop Plants*, Volume 2. Springer, New York, NY, pp. 381–409. https://doi.org/10.1007/978-1-4614-9572-7_16.
- Sagare, D.B., Mohanty, I.C., 2012. Development of moisture stress tolerant brinjal cv. Utkal Anushree (*Solanum melongena* L.) using Agrobacterium mediated gene transformation. *J. Agric. Sci.* 4, 141.
- Saiki, Y., Uchimura, Y., Nakahara, T., 2009. A study for increased rates in plant regeneration from eggplant [Aubergines] (*Solanum melongena* L.) microspores. *Bull. Fukuoka Agric. Res. Center (Japan)*.
- Saito, T., Nishimura, S., 1994. Improved culture conditions for somatic embryogenesis using an aseptic ventilative filter in eggplant (*Solanum melongena* L.). *Plant Sci.* 102, 205–211.
- Sarker, R.H., Yesmin, S., Hoque, M.I., 2006. Multiple shoot formation in eggplant (*Solanum melongena* L.). *Plant Tissue Culture Biotechnol.* 16, 53–61.
- Scutt, C.P., Zubko, E., Meyer, P., 2002. Techniques for the removal of marker genes from transgenic plants. *Biochimie* 84, 1119–1126.
- Sharma, P., Rajam, M.V., 1995. Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). *J. Exp. Bot.* 46, 135–141. <https://doi.org/10.1093/jxb/46.1.135>.
- Sharma, H.C., Crouch, J.H., Sharma, K.K., Seetharama, N., Hash, C.T., 2002. Applications of biotechnology for crop improvement: prospects and constraints. *Plant Sci.* 163, 381–395.
- Shivaraj, G., Rao, S., 2010. In vitro organogenesis and plant regeneration in eggplants (*Solanum melongena* L.). *Curr. Trends Biotechnol. Pharm.* 4, 842–849.
- Sidhu, M.K., Dhatt, A.S., Sandhu, J.S., Gosal, S.S., 2014a. Biolistic transformation of cry 1Ac gene in eggplant (*Solanum melongena* L.). *Int. J. Agric. Environ. Biotechnol.* 7, 679.
- Sidhu, M.K., Dhatt, A.S., Sidhu, G.S., 2014b. Plant regeneration in eggplant (*Solanum melongena* L.): a review. *Afr. J. Biotechnol.* 13, 714–722. <https://doi.org/10.5897/AJBX2013.13521>.
- Singh, A.K., Verma, S.S., Bansal, K.C., 2010. Plastid transformation in eggplant (*Solanum melongena* L.). *Transgenic Res.* 19, 113–119.
- Singh, D., Haicour, R., Sihachakr, D., Rajam, M.V., 2015. Expression of rice chitinase gene in transgenic eggplant confers resistance to fungal wilts. *Indian J. Biotechnol.* 14, 233–240.
- Singh Yadav, J., Venkat Rajam, M., 1998. Temporal regulation of somatic embryogenesis by adjusting cellular polyamine content in eggplant. *Plant Physiol.* 116, 617–625.
- Smith, R.H., 2013. *Plant Tissue Culture: Techniques and Experiments*. Academic Press.
- Song, Y., Shen, S., Wang, Y., Chen, X., Luo, S., 2007. Studies on microspore culture of eggplant (*Solanum melongena* L.) obtained callus [J]. *J. Agric. Univ. Hebei* 3, 006.
- Soniya, E.V., Kannapiran, P., Unni, S.C., Joy, N., Radhakrishnan, E.K., 2005. Transformation and regeneration of *Solanum melongena* using *Agrobacterium tumefaciens*. *J. Plant Biol.* – New Delhi 32, 29.
- Soyk, S., Müller, N.A., Park, S.J., Schmalenbach, I., Jiang, K., Hayama, R., et al., 2017. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nat. Genet.* 49, 162–168. <https://doi.org/10.1038/ng.3733>.
- Sree, K.S., Rajam, M.V., 2015. Genetic engineering strategies for biotic stress tolerance in plants. *Plant Biology and Biotechnology*. Springer, New Delhi, pp. 611–622. https://doi.org/10.1007/978-81-322-2283-5_30.
- Suginiura, Y., Miyazaki, J., Yonebayashi, K., Kotani, E., Furusawa, T., 1999. Gene transfer by electroporation into protoplasts isolated from mulberry calli. *J. Sericultural Sci. Jap.* 68, 49–53.
- Sunseri, F., Fiore, M.C., Mastrovito, F., Tramontano, E., 1993. In vivo selection and genetic analysis for kanamycin resistance in transgenic eggplant (*Solanum melongena* L.). *J. Genet. Breeding* 47, pp. 299–299.
- Swamynathan, B., Nadanakunjidam, S., Ramamouri, A., Sindhu, K., Ramamoorthy, D., 2010. In-vitro plantlet regeneration through somatic embryogenesis in *Solanum melongena* (Thengaitthittu Variety). *Academic J. Plant Sci.* 3, 64–70.
- Taghipour, F., Jamshidi, S., Huyop, F., 2015. High performant eggplant in vitro regeneration and organogenesis. *Agroecol. J.* 11, 24–29.
- Taher, D., Solberg, S.O., Prohens, J., Chou, Y., Rakha, M., Wu, T., 2017. World vegetable center eggplant collection: origin, composition, seed dissemination and utilization in breeding. *Front. Plant Sci.* 8. <https://doi.org/10.3389/fpls.2017.01484>.
- Tanaka, H., Johkan, M., Mitsukuri, K., Tezuka, T., Furukawa, H., Oda, M., 2013. Intact roots promote shoot regeneration from hypocotyl independent of exogenous plant growth regulators in eggplant in vitro. *Plant Root* 7, 5–11.
- Tarré, E., Magioli, C., Margis-Pinheiro, M., Sachetto-Martins, G., Mansur, E., Santiago-Fernandes, L.D., 2004. In vitro somatic embryogenesis and adventitious root initiation have a common origin in eggplant (*Solanum melongena* L.). *Braz. J. Bot.* 27, 79–84.
- Thomazella, D.P.de T., Brail, Q., Dahlbeck, D., Staskawicz, B.J., 2016. CRISPR-Cas9 mediated mutagenesis of a DMR6 ortholog in tomato confers broad-spectrum disease resistance. *bioRxiv* 064824. <https://doi.org/10.1101/064824>.
- Toppino, L., Kooiker, M., Lindner, M., Dreni, L., Rotino, G.L., Kater, M.M., 2011. Reversible male sterility in eggplant (*Solanum melongena* L.) by artificial microRNA-mediated silencing of general transcription factor genes. *Plant Biotechnol. J.* 9, 684–692.
- Twyman, R.M., Christou, P., 2004. Plant transformation technology – particle bombardment. In: In: Christou, P., Klee, H. (Eds.), *Handbook of Plant Biotechnology*, vol. 1. John Wiley & Sons, NY, pp. 263–290.
- Van Eck, J., 2018. Genome editing and plant transformation of solanaceous food crops. *Curr. Opin. Biotechnol.* 49, 35–41. <https://doi.org/10.1016/j.copbio.2017.07.012>.
- Vasil, I.K., Thorpe, T.A., 2013. *Plant Cell and Tissue Culture*. Springer Science & Business Media.
- Xing, Y., Yu, Y., Luo, X., Zhang, J.-N., Zhao, B., Guo, Y.-D., 2010. High efficiency organogenesis and analysis of genetic stability of the regenerants in *Solanum melongena*. *Biol. Plantarum* 54, 231–236.
- Yamada, T., Nakagawa, H., Sinoto, Y., 1967. Studies on differentiation in cultured cells. I. Embryogenesis in 3 strains of solanum callus. *Botanical Mag.-Tokyo* 80, 68.
- Yang, Y., Li, R., Qi, M., 2000. In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. *Plant J.* 22, 543–551. <https://doi.org/10.1046/j.1365-3113.2000.00760.x>.
- Yesmin, S., Khatun, M.M., Tanny, T., Protity, A.T., Salimullah, M., Alam, I., 2018. In vitro regeneration of two high-yielding eggplant (*Solanum melongena* L.) varieties of Bangladesh. *Curr. Bot.* 08–12.
- Zayova, E., Vassilevska-Ivanova, R., Kraptchev, B., Stoeva, D., 2012. Indirect shoot organogenesis of eggplant (*Solanum melongena* L.). *J. Central Eur. Agric.* 13, 446–457.
- Zhang, M., Chen, Y., Liu, F., Zhang, Y., Lian, Y., 2014. Optimization on regeneration protocol for genetic transformation of eggplant. *J. Changjiang Vegetables* 14, 004.
- Zhao, H., Tan, Z., Wen, X., Wang, Y., 2017. An improved syringe agroinfiltration protocol to enhance transformation efficiency by combinative use of 5-azacytidine, ascorbate acid and Tween-20. *Plants* 6, 9.
- Zulkarnain, Z., Tapingkae, T., Taji, A., 2015. Applications of in vitro techniques in plant breeding. *Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools*. Springer, pp. 293–328.